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Effects of different humic substances concentrations on root anatomy and Cd accumulation in seedlings of *Avicennia germinans* (black mangrove)

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ABSTRACT

Mangrove areas are among most threatened tropical ecosystems worldwide. Among polluting agents Cadmium is often found in high concentrations in mangrove sediments. Humic substances, complex biomolecules formed in soil and sediments during animal and plant residuals decomposition, have a known biostimulant activity and can be adopted to counteract various plant stresses. This study explores, in controlled conditions, the effect of humic substances on *Avicennia germinans* seedlings, with or without cadmium contamination. Humic compounds significantly changed plant root architecture, and, when coupled with cadmium, root anatomy and Cortex to Vascular Cylinder diameter ratio. These modifications led to lower Cd uptake by humic substances-treated plants. Humic substances amendment could be effective, depending on their concentrations, on improving plant health in mangrove areas, for forest recuperation and/or dredged sediments phytoremediation purposes.

1. Introduction

Mangrove forests, the intertidal wetlands confined to tropical and subtropical areas, are special ecosystems with a high level of biodiversity (Field et al., 1998). Mangrove play a key role in the conservation of tropical and subtropical coastlines, supporting a wide variety of ecosystem services (Atkinson et al., 2016) including nutrient cycling, soil formation, wood production, ecotourism. These areas also provide critical nurseries and habitats for fish and crustacean (Lee et al., 2014) which can pass all their life cycle or part of it inside the mangrove. Additionally, containing on average 1023 Mg carbon per hectare, mangroves are among the most carbon-rich forests in the tropics (Donato et al., 2011), thus the conservation of carbon-rich mangroves should be a high-priority component of strategies to mitigate climate change (Atwood et al., 2017). Approximately 150,000 km^2 of mangroves exist worldwide, over two thirds of the forests are located in just eighteen countries among which Indonesia, Brazil and Australia are the top three (Barbier, 2016).

Mangroves are also one of the most threatened tropical ecosystems worldwide, as a consequence of increasing pollution from human activities due to rapid industrialization and urbanization of coastal regions: as a result, the areal extent of mangrove forests has declined by 30–50% over the past half century (FAO, 2007).

Among degradation factors of mangroves forests there is the accumulation of pollutants trough rivers and harbor activities that are often placed close to them (Borja et al., 2012). Pollutants are often illegally discarded on the rivers and consequently on mangroves forests (Maiti and Chowdhury, 2013) producing polluted sediments, often insisting inside the mangrove ecosystems for many years (Bayen, 2012; Bortone et al., 2004; Le Guyader, 2013). This causes significant forest decline, leaving areas characterized by several spots without canopy, as it happening for example, in the mangrove area of Maria Ortiz, a neighborhood of Vitoria, capital city of Espirito Santo State, Brazil (Dobbss, 2012). Moreover, mangrove species might be adopted for the decontamination of the polluted sediments produced from harbor sediment dredging (Pittarello et al., 2017).

Within different classes of pollutants, heavy metals are not readily degradable in nature. Sediments in the mangrove areas represent important natural sinks for heavy metals, involved in the regulation of metal distribution (Chakraborty et al., 2014). However, the total metal

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concentration in sediments is not necessarily an indication for their fate, dispersal and bioavailability. Bioavailability in sediments depends on their chemical speciation which is influenced by buffer capacity of water soluble and exchangeable metal complexes, organic matter and sulphides (Chakraborty et al., 2015a). Metals concentration in mangroves root system is considered as an indicator of metals bioavailability (Chakraborty et al., 2014). There are four typical Brazilian woody mangrove species: *Rizophora mangle*, *Laguncularia racemosa*, *Avicennia germinans* and *Avicennia shaueriana* (Tomlinson, 1986). Woody mangroves species, when able to grow in their own ecosystem, are effective in heavy metal phytostabilization trough metal precipitation in rhizosphere and/or bioaccumulation in roots (MacFarlane et al., 2007), allowing the "self-preservation" of this ecosystem. Some authors showed an interesting potential of *Avicennia* spp. in heavy metals accumulation and translocation to stem (MacFarlane et al., 2003; González-Mendoza et al., 2007). Souza et al. (2015) found that *Avicennia shaueriana* tries to translocate more trace elements to stem and leaves in case of high accumulation in roots, probably to avoid an excess of toxicity in roots tissues. In general, *Avicennia* spp. seems to be promising in sediment phytoremediation, also due to their attitude to exclude salt trough leaves glands: this characteristic leads to a weaker thickening of exodermis cell walls in comparison with other mangroves (Pittarello et al., 2017). Moreover, roots of mangrove species can modify the surrounding sediments redox conditions with oxygen supplied to rhizosphere by aerenchyma (Jacob and Otte, 2003). This influences bioavailability of Cd, which, in surface sediments, is mobile under oxidizing conditions at pH levels below 8 (Chakraborty et al., 2015b).

Aerenchyma is a spongy tissue present in root mangrove aerial extensions termed 'pneumatophores' and in absorbing roots (Hogarth, 1999). These tissues guarantee oxygen supply in anaerobic substrates ensuring O_2 diffusion into below ground root portion (Youssef and Saenger, 1996). The part of this O_2 stored inside the roots that exceeds the need for aerobic respiration, is released through the aerenchyma into the surrounding rhizosphere. This mechanism is called radial oxygen loss (ROL) in wetland plants (Armstrong, 1978; Colmer and Pedersen, 2008). In case of trace elements contamination several mangroves species like *Aegiceras corniculatum*, *Bruguiera gymnorrhiza* and *Rhizophora stylosa*, increase the thickness of outer cortex and lignification in cell walls with the consequence to reduce ROL (Cheng et al., 2010). Pi et al. (2009) found that *Avicennia marina* roots present the thinner outer cortex both in mature zone, 8 cm from root tip, and close to the tip, in comparison with other seven mangrove species, although shows the highest resistance to waterlogging. Souza et al. (2015) found root anatomical changes in *Avicennia shaueriana* in four mangrove forests of Espirito Santo State (Brazil), characterized by different levels of oxygen deficiency in sediments: seems that in *A. shaueriana* pneumatophores and absorbing roots cortex and aerenchyma area were inversely correlated with oxygen concentration in sediments.

Cadmium (Cd) is a non-essential heavy metal, released into the environment by traffic or industrial activities like mining, electroplating, manufacturing of plastics, alloy preparation. It also derives from batteries that contain Cd and it is a by-product of mineral fertilizers. Cd is considered highly toxic for animals and humans due to its solubility in water depending on its speciation. In particular, due to their longevity, humans can accumulate Cd in their organs by eating contaminated plants and animals (Kirkham, 2006): for this it is important to implement all possible actions in order to remove Cd from food chain or block its circulation in soil and water (Grant et al., 1998). In humans Cd intoxication can lead to kidney, skeletal, respiratory and reproductive systems damages (Godt et al., 2006).

While Cd can sometimes be found in high concentrations in mangrove sediments (Nascimento et al., 2006), bioaccumulation of Cd in edible animals can be high in mangrove systems even at low Cd loading in the sediment (Chakraborty et al., 2015b; Chakraborty et al., 2015a).

In plants Cd exerts its toxic effects competing with several essentials nutrients and/or impairing transportation mechanisms: its competition with Fe causes chlorosis (Das et al., 1997), it decreases water content (Sanita di Toppi and Gabrielli, 1999) and causes root tips browning with a consequent growth inhibition (Kahle, 1993). Interestingly, as found by Baryla et al. (2001) in *Brassica napus*, Cd does not directly impair antenna photosystem but decreases number of chloroplasts per cell and changes cell size, suggesting that Cd interferes with chloroplast replication and cell division. Furthermore, although brassicas grew in highly enriched CO_2 atmosphere ($4000\,\mu L\,CO_2\,l^{-1}$), growth inhibition by Cd was not reversed, probably because low stomatal conductance was not the main effect of Cd toxicity. First one of these two insights are confirmed by Di Cagno et al. (1999) who evidenced that in *Heliantus annus* seedlings treated with Cd^{2+} the photochemical efficiency of photosystem II (PSII) was not altered although, after 7 d of treatment leaf area, chlorophyll content and CO_2 assimilation rate decreased both in young and mature leaves. Water deficit was clear in *Heliantus annus* exposed to excess concentrations of Cd (Kastori et al., 1992). In *Cajanus cajan*, 20 mM Cd^{2+} inhibited by 87% CO_2 exchange rate and the extent of inhibition increased with duration of exposure. Stomatal conductance decreased in parallel with transpiration rate. After 10 days of treatment, wilting occurred (Sheoran et al., 1990). Costa and Morel (1994) found that in hydroponic, Cd above 100 μM caused stomatal closure in lettuce, while the opposite effect was obtained with concentrations up to 0.1 μM.

Cd is mainly accumulated in roots but depending on plant species, plant organs, its concentration and time to exposure, it can be significantly translocated to shoots and use same N and P pathway to reach shoots and reproductive organs. In wheat (*Triticum turgidum*) Cd accumulation in grains increased significantly with N and P applications, indicating an environmental effect on Cd phytoavailability (Grant and Bailey, 1998; Di Cagno et al., 1999). On the opposite, in *Heliantus annus* Simon (1998) evidenced N, P, K, Ca, Mg, Cu, Fe, Mn and Zn uptake was not influenced by lower or higher Cd concentrations; Cd accumulation in roots shoots and leaves was directly correlated with its concentration in the medium and the main accumulation was in roots, up to $13.69\,mg\,kg^{-1}$ depending on the concentration. In aquatic species, *Bacopa monnieri* maximum Cd accumulation ($906.5\,mg\,kg^{-1}$ DW) was found in roots exposed to 200 μM Cd for 144 h (Singh et al., 2006).

Cd attitude to being accumulated in plants roots and reproductive organs can be a great risk for animal and human health, however, several woody species can be successfully adopted in Cd-contaminated soil to accumulate the metal in the plant organs aiming to soil phytoremediation. For example Robinson et al. (2000) employed poplar and willow clones in soils containing a range of Cd concentrations among 0.6 and $60.6\,mg\,kg^{-1}$ dry soil obtaining a Cd accumulation in willows clones up to $167\,mg\,kg^{-1}$ while poplars accumulated up to $209\,mg\,kg^{-1}$. *Avicennia marina,* in Cd low contaminated sediment ($1.23\,mg\,kg^{-1}$) achieved to translocate to leaves $1.04\,mg\,kg^{-1}$ (Usman et al., 2013).

Phytoremediation efficiency is affected by multiple environmental factors, such as soil texture, pH, redox conditions, cation exchange capacity, microorganisms, Ca/Fe/Mn/Al/P contents and the presence of natural organic matter (Pittarello et al., 2017). Within natural organic matter, humic substances (HS) are known to affect both plant physiology (Nardi et al., 2009) and metal availability. In particular, environmental availability of metals can be increased or decreased by exogenous humic substances in relationship with metal, pH and soil characteristics (Wiszniewska et al., 2016): Chakraborty (2010) reported that at $pH < 6$ humic acids are aggregated due to neutralization of negative charges by H^+ leaving Cd mainly in bioavailable form in the solution; at $pH > 6$ humic acids disaggregate offering more complexing sites for Cd^{2+} ions forming inert complexes when pH increases up to 7.

Cd-humic matter interaction have several other consequences: Cd can be found as weak complexes with dissolved or sedimentary organic